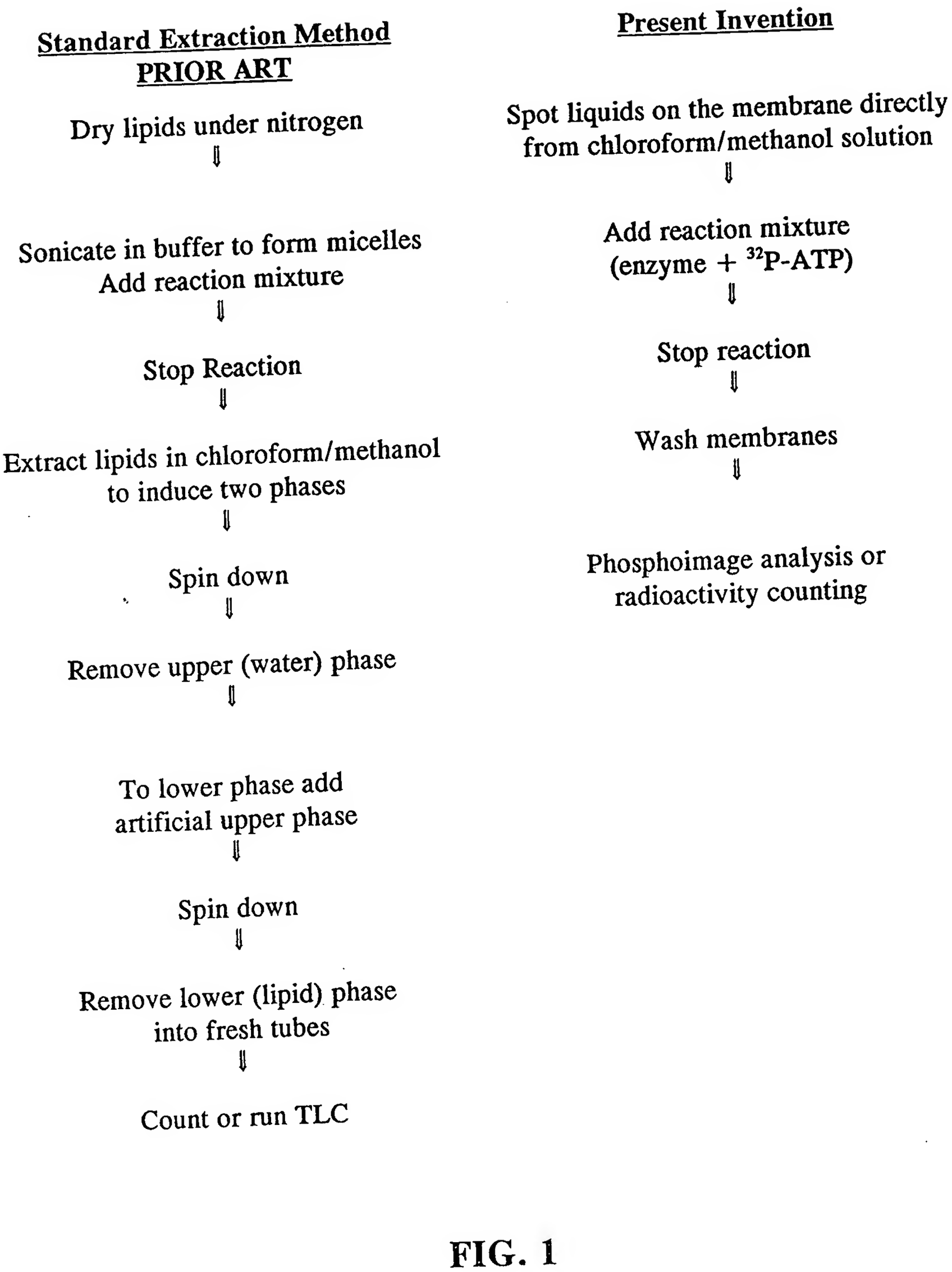
Standard Extraction Method
PRIOR ART
Dry lipids under nitrogen
Sonicate in buffer to form micelles
Add reaction mixture
Stop Reaction
Extract lipids in chloroform/methanol
to induce two phases
Spin down
Remove upper (water) phase
To lower phase add
artificial upper phase
Spin down
Remove lower (lipid) phase
into fresh tubes
Count or run TLC
Present Invention
Spot liquids on the membrane directly
from chloroform/methanol solution
Add reaction mixture
(enzyme + 32P-ATP)
Stop reaction
Wash membranes
Phosphoimage analysis or
radioactivity counting

FIG. 1

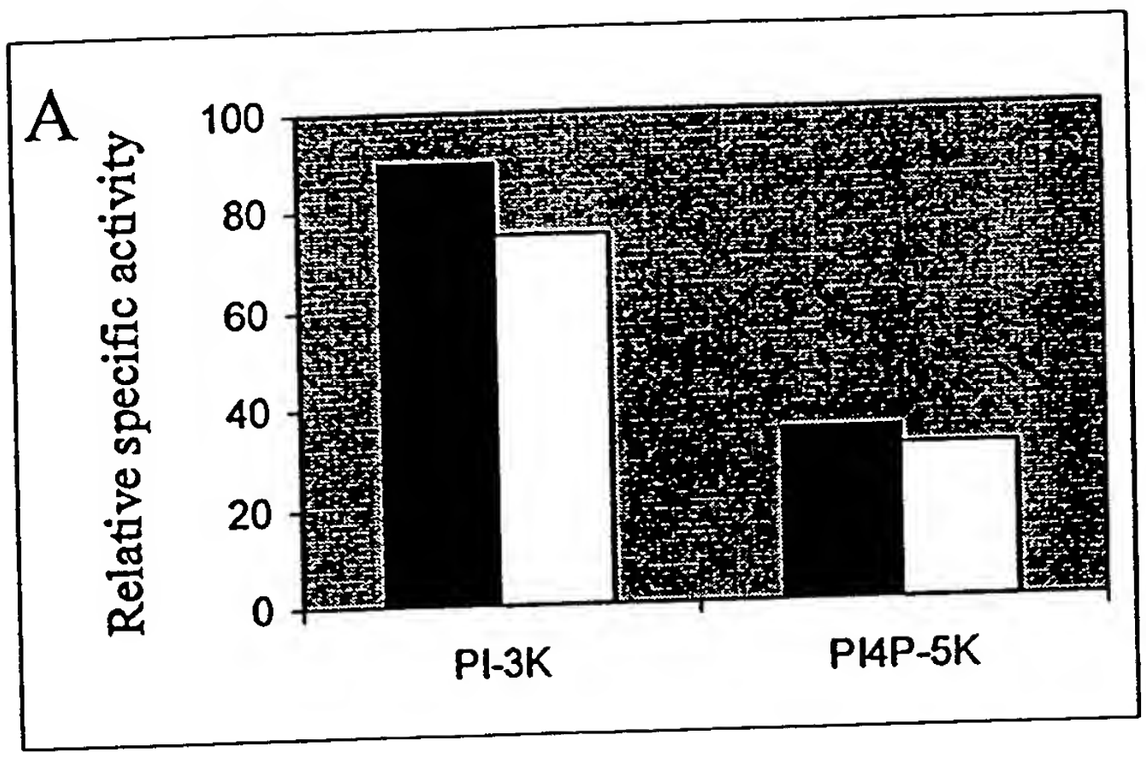
A
Relative specific activity
100
80
60
40
20
0
PI-3K
PI4P-5K

FIG. 2A

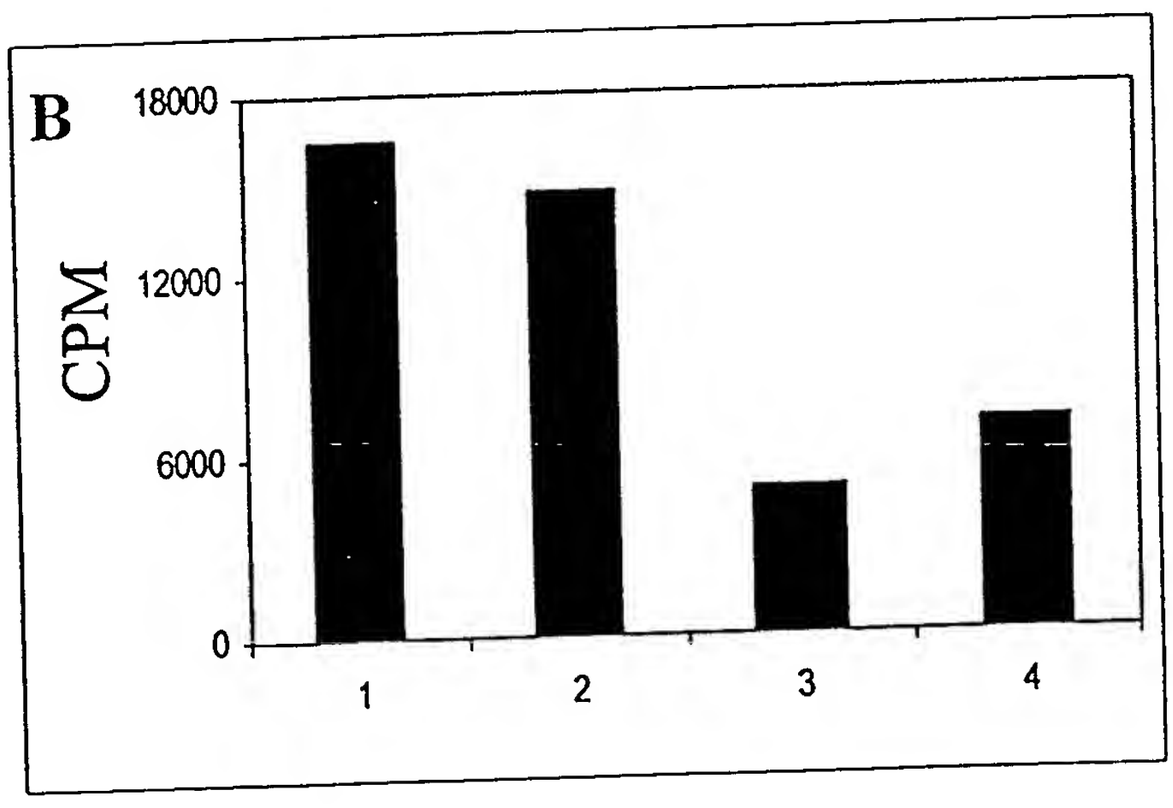
B
CPM
18000
12000
6000
0
1
2
3
4

FIG. 2B

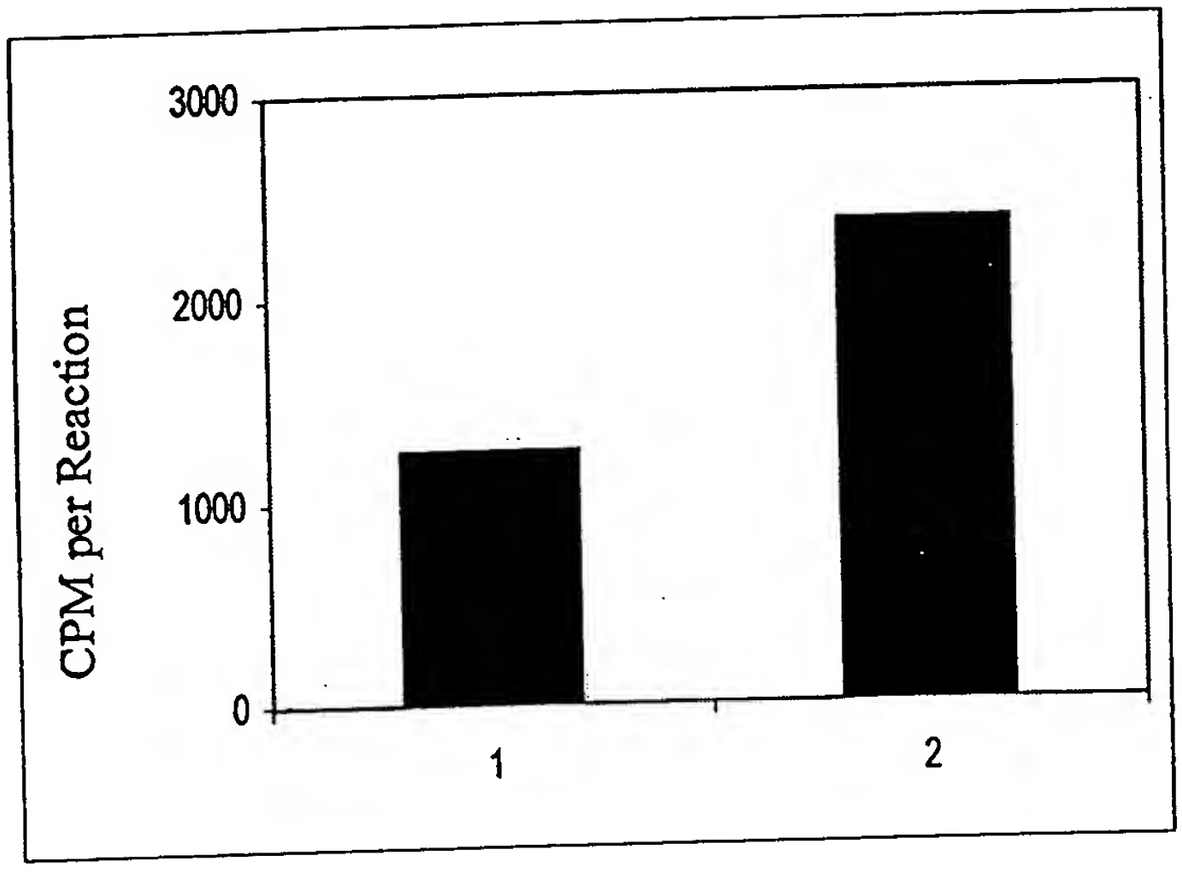

FIG. 3

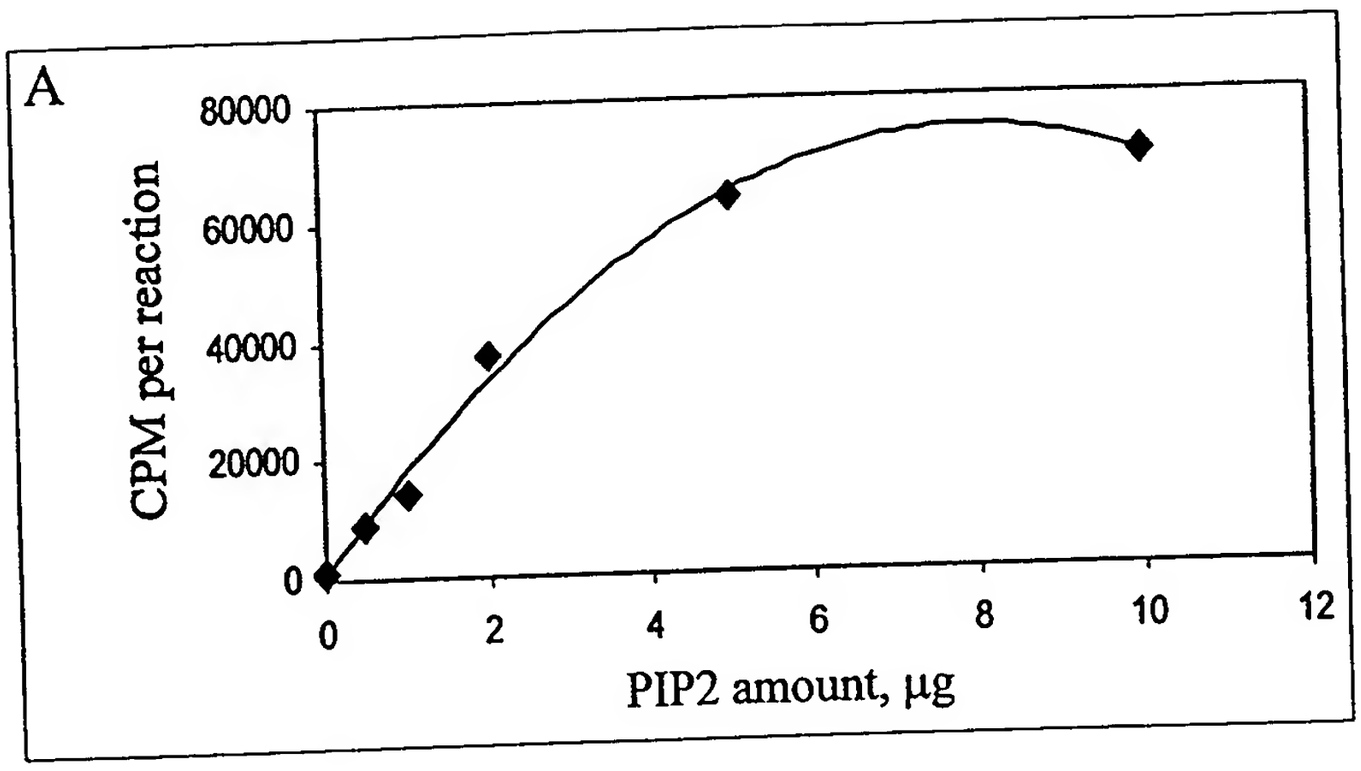
A
80000
60000
40000
20000
0
CPM per reaction
0
2
4
6
8
10
12
PIP2 amount, µg

FIG. 4A

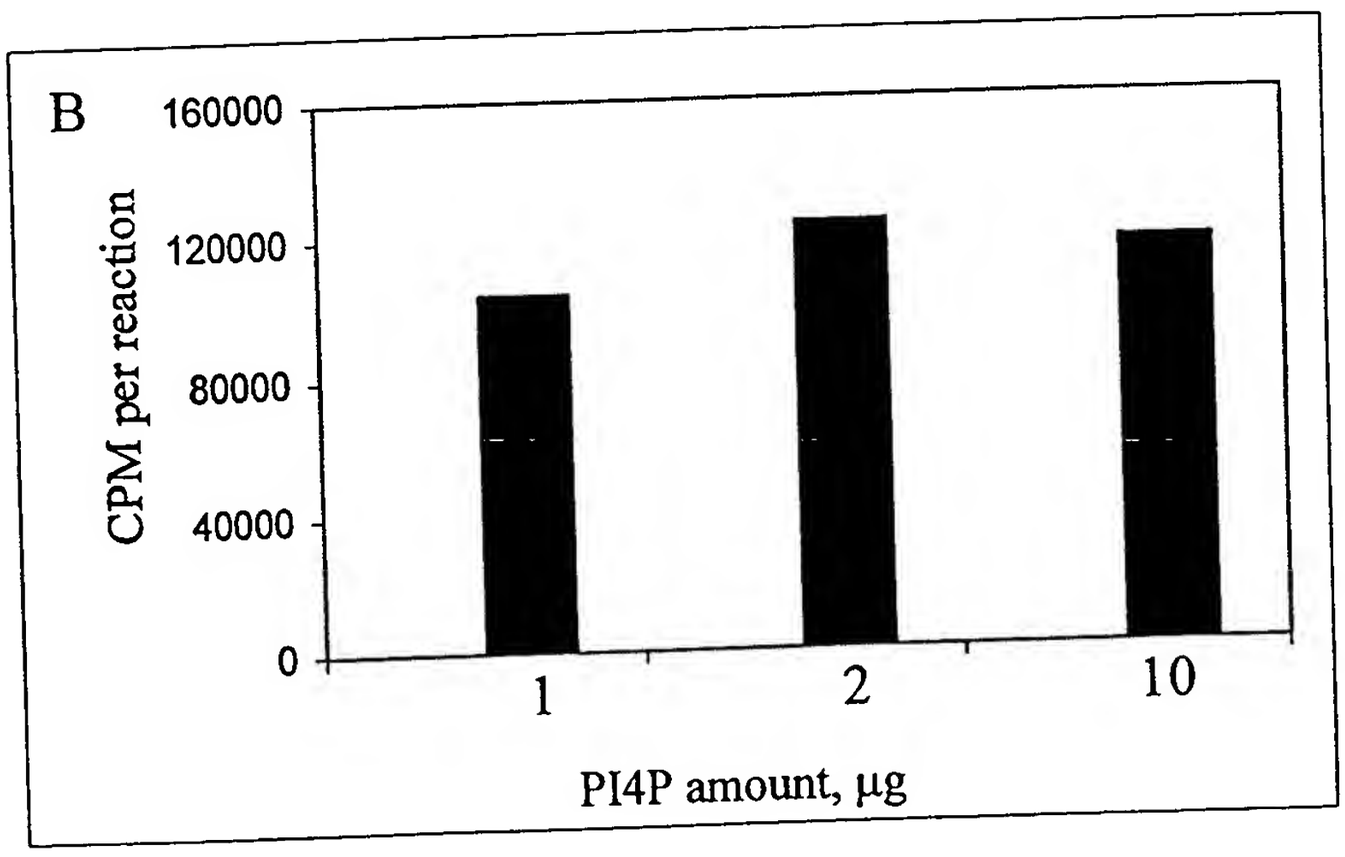
B
160000
120000
80000
40000
0
CPM per reaction
1
2
10
PI4P amount, µg

FIG. 4B

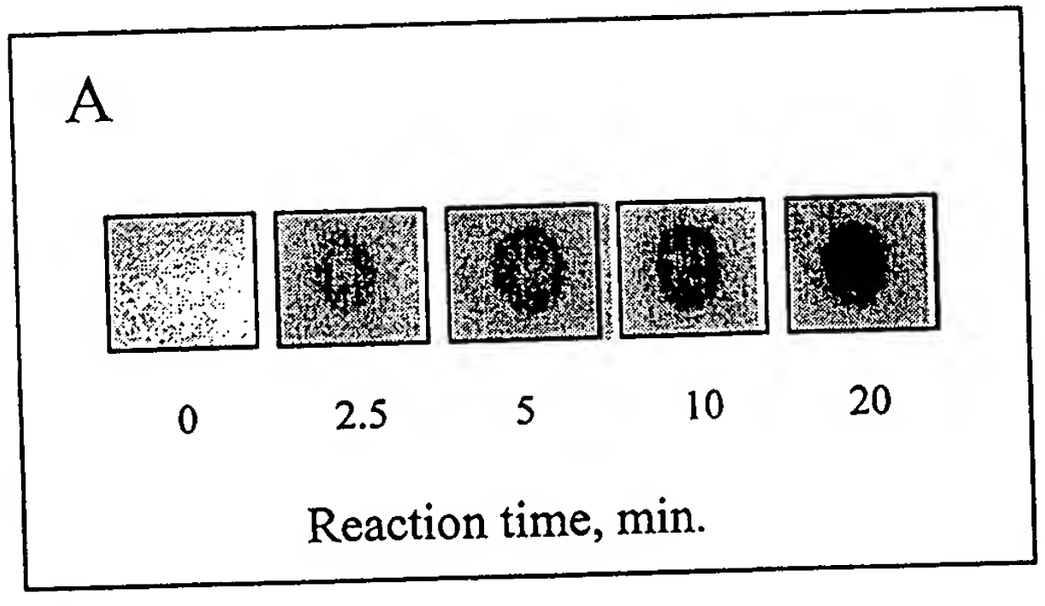

FIG. 5A

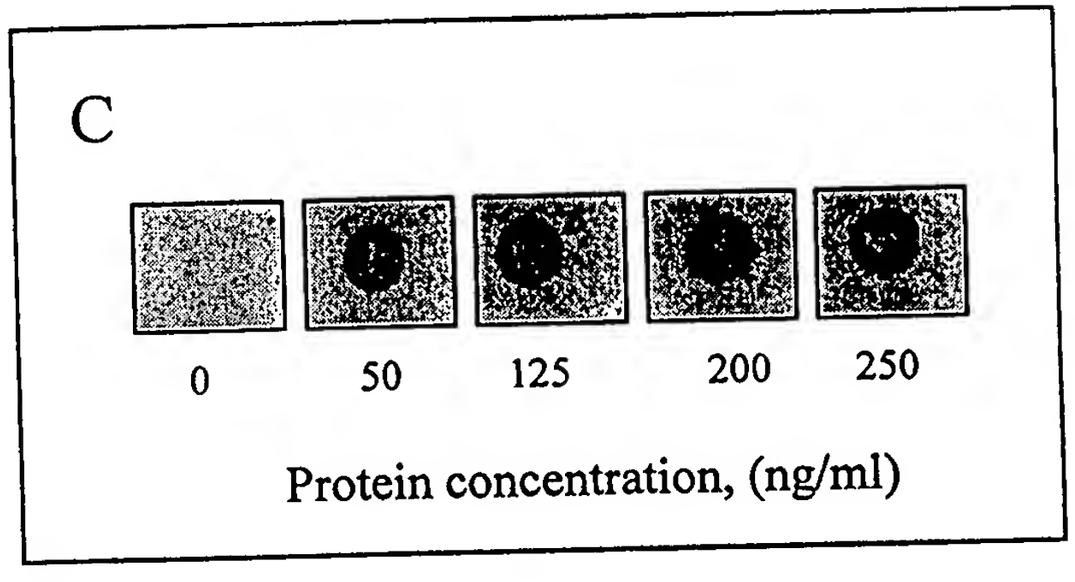

FIG. 5C

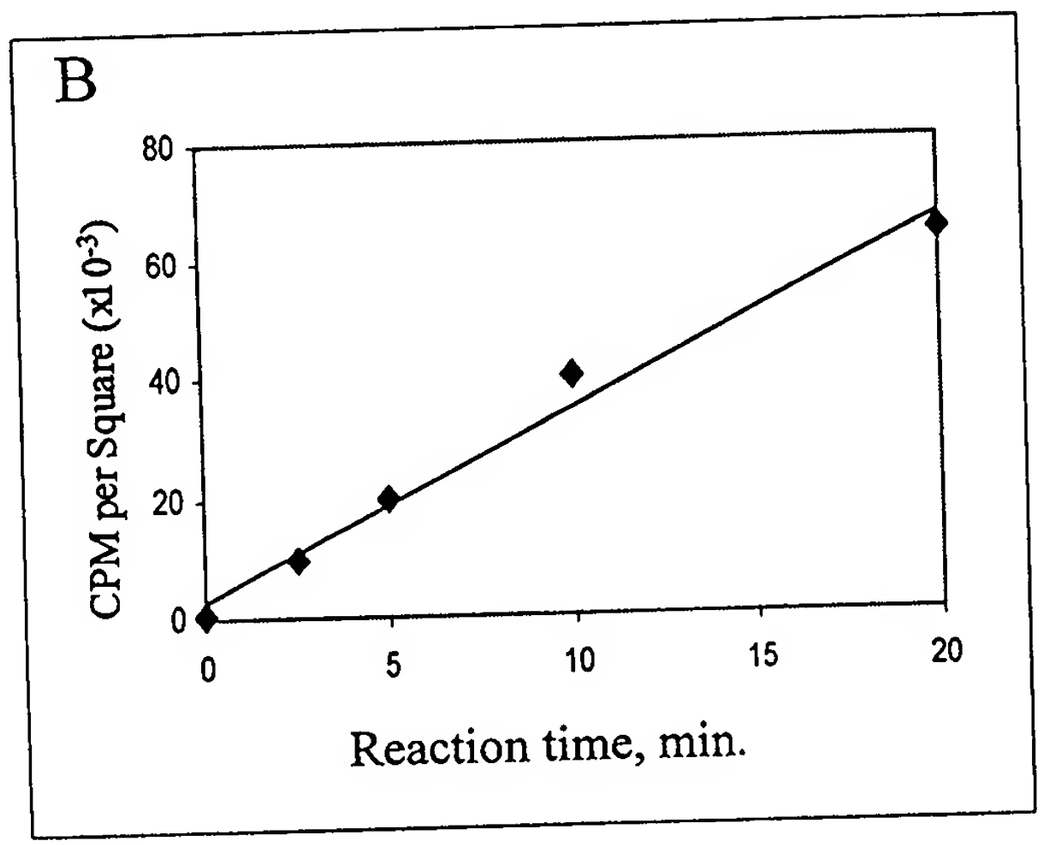

FIG. 5B

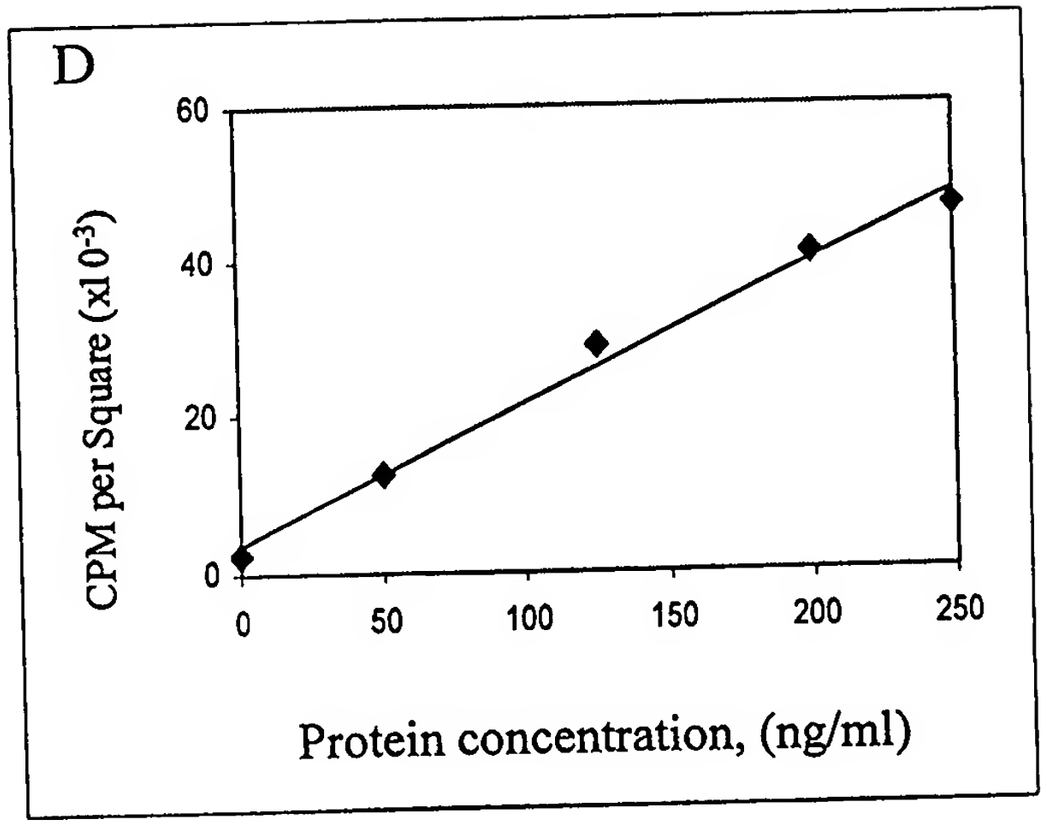

FIG. 5D

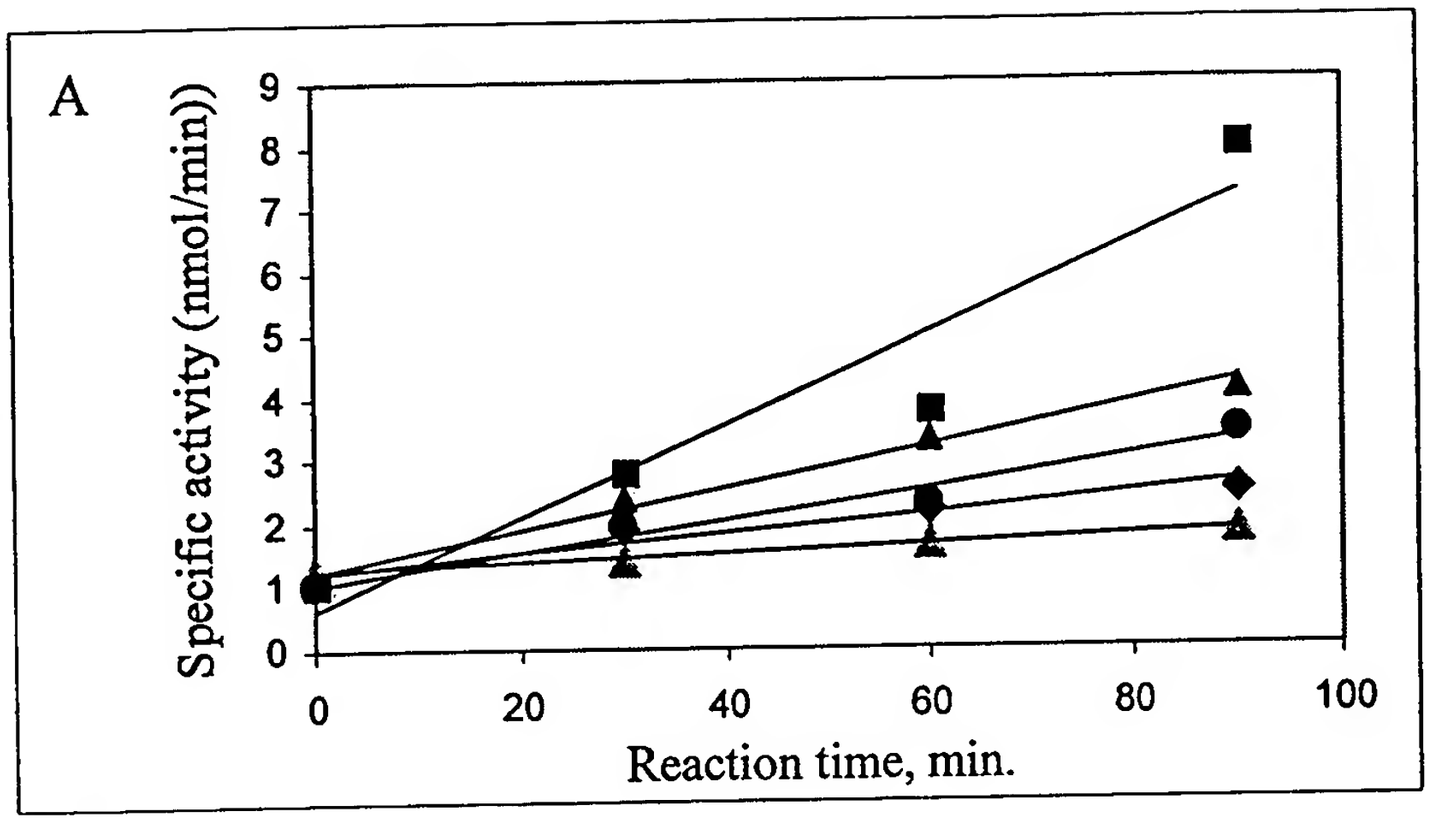
A
Specific activity (nmol/min))
0
1
2
3
4
5
6
7
8
9
0
20
40
60
80
100
Reaction time, min.

FIG. 6A

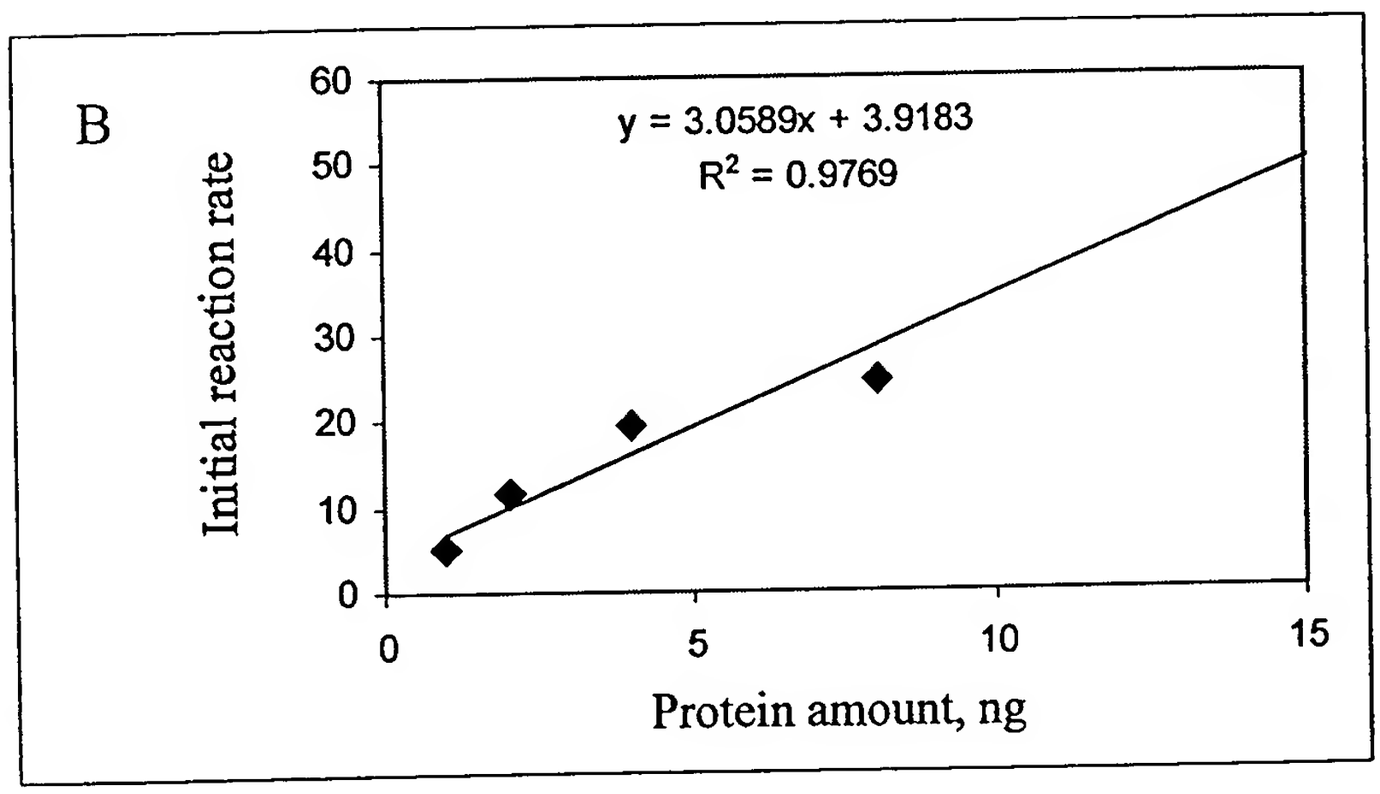
B
Initial reaction rate
y = 3.0589x + 3.9183
R2 = 0.9769
0
10
20
30
40
50
60
0
5
10
15
Protein amount, ng

FIG. 6B

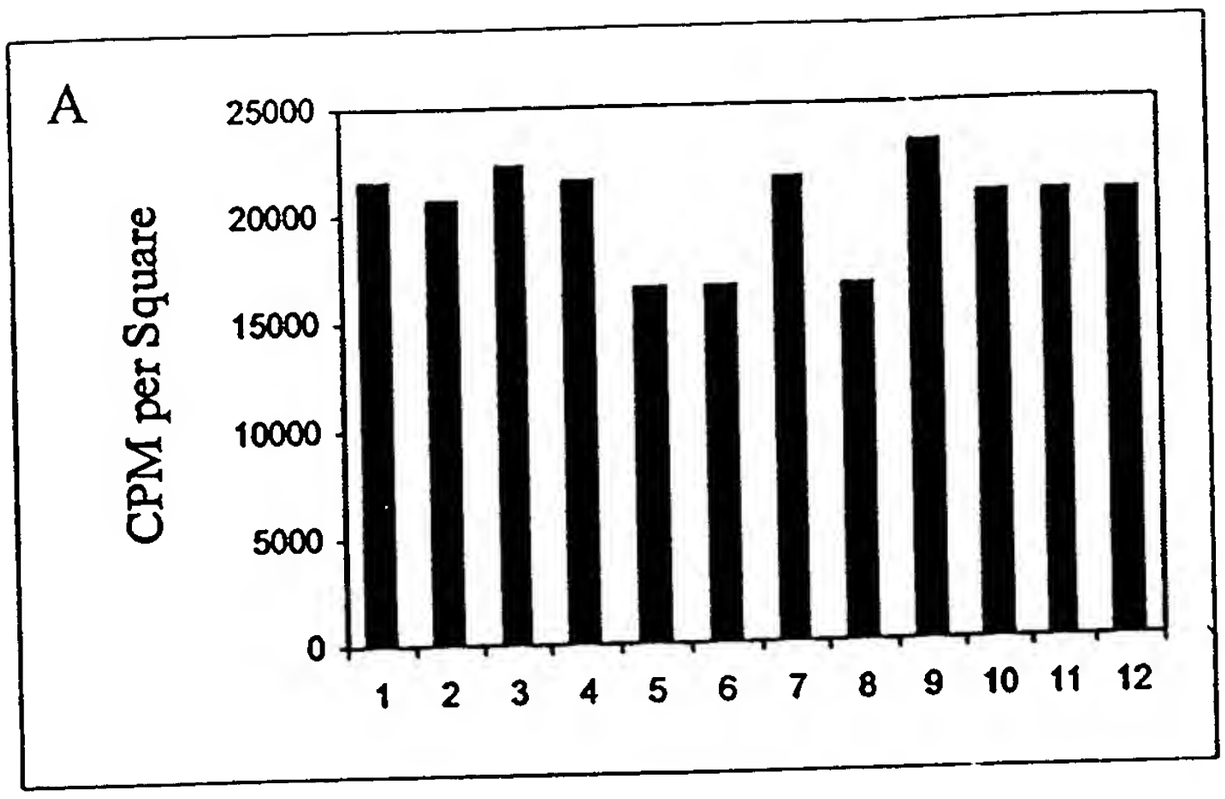

FIG. 7A

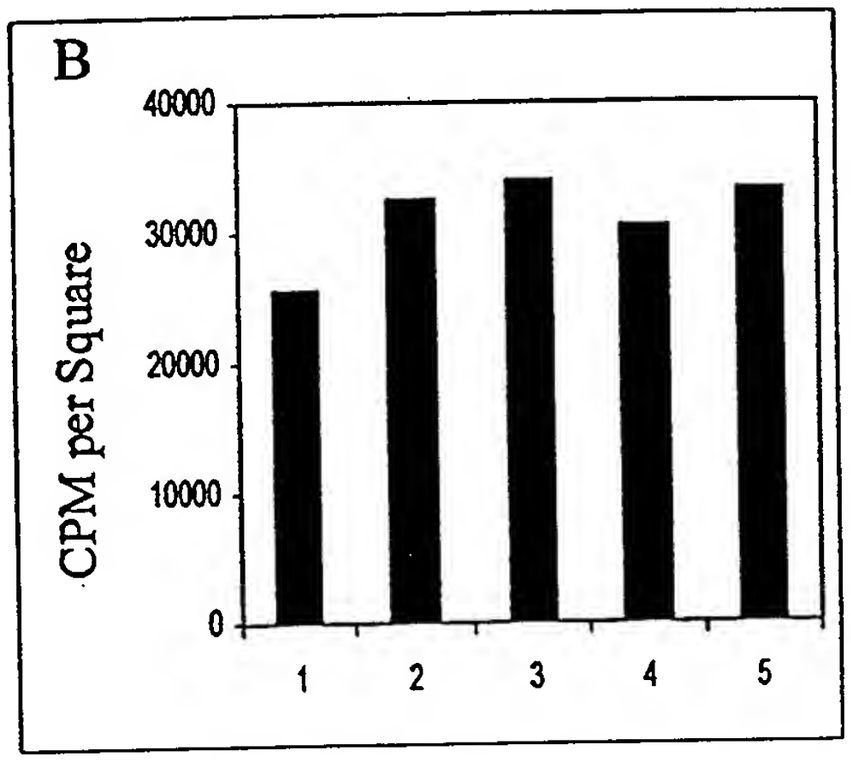

FIG. 7B

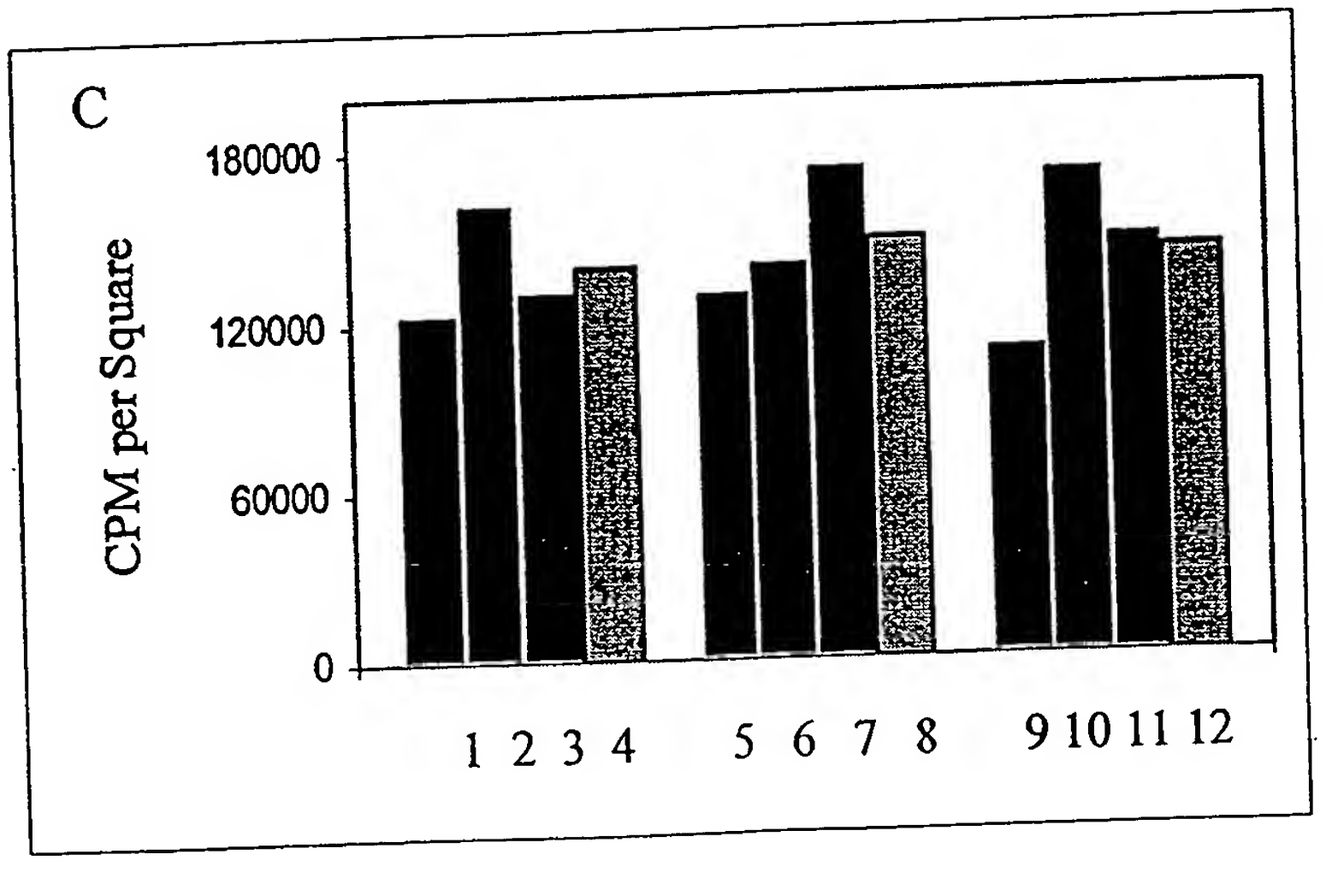

FIG. 7C

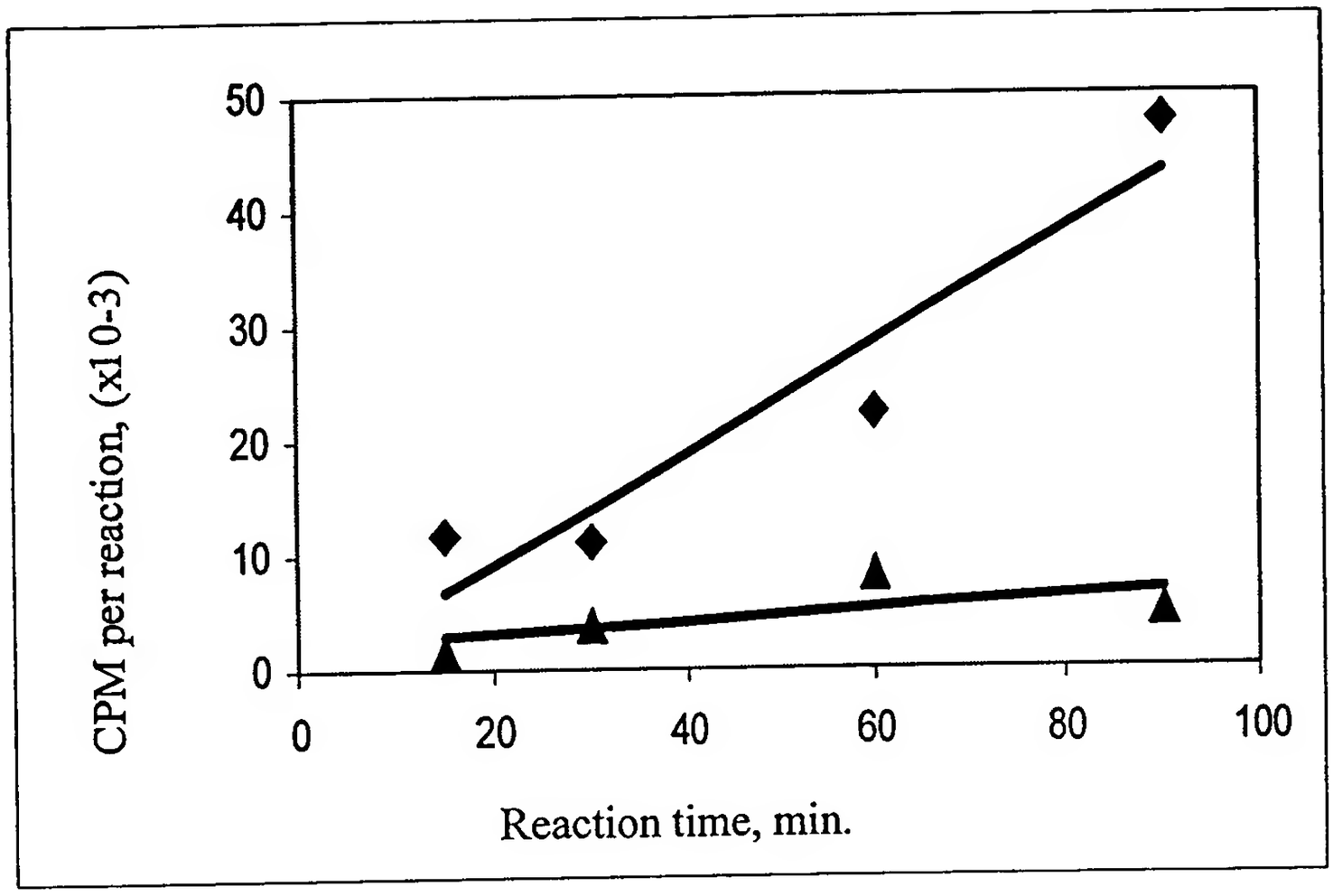
50
40
30
20
10
0
0
20
40
60
80
100
CPM per reaction, (x1 0-3)
Reaction time, min.

FIG. 8

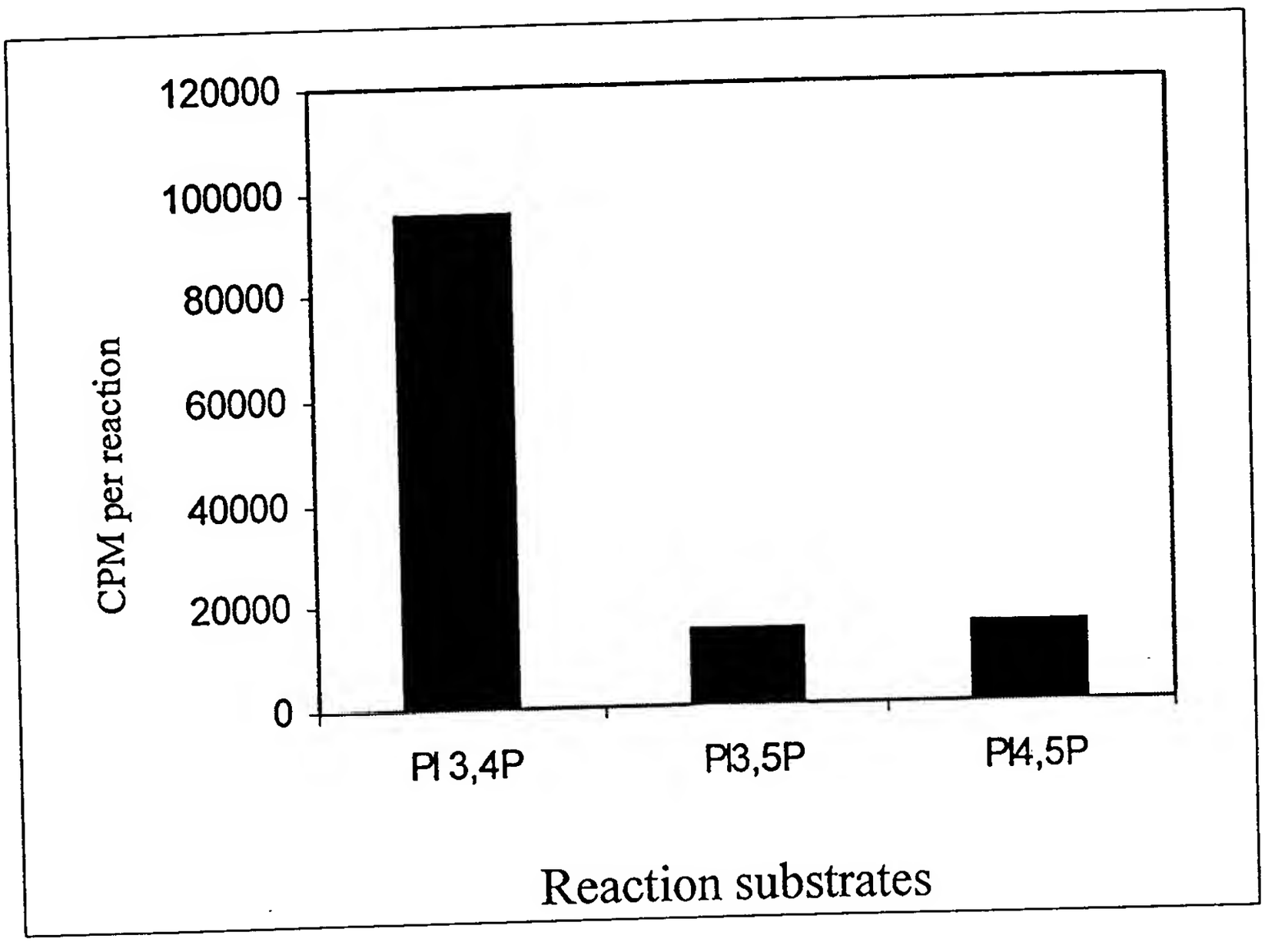
120000
100000
80000
60000
40000
20000
0
CPM per reaction
PI 3,4P
PI3,5P
PI4,5P
Reaction substrates

FIG. 9

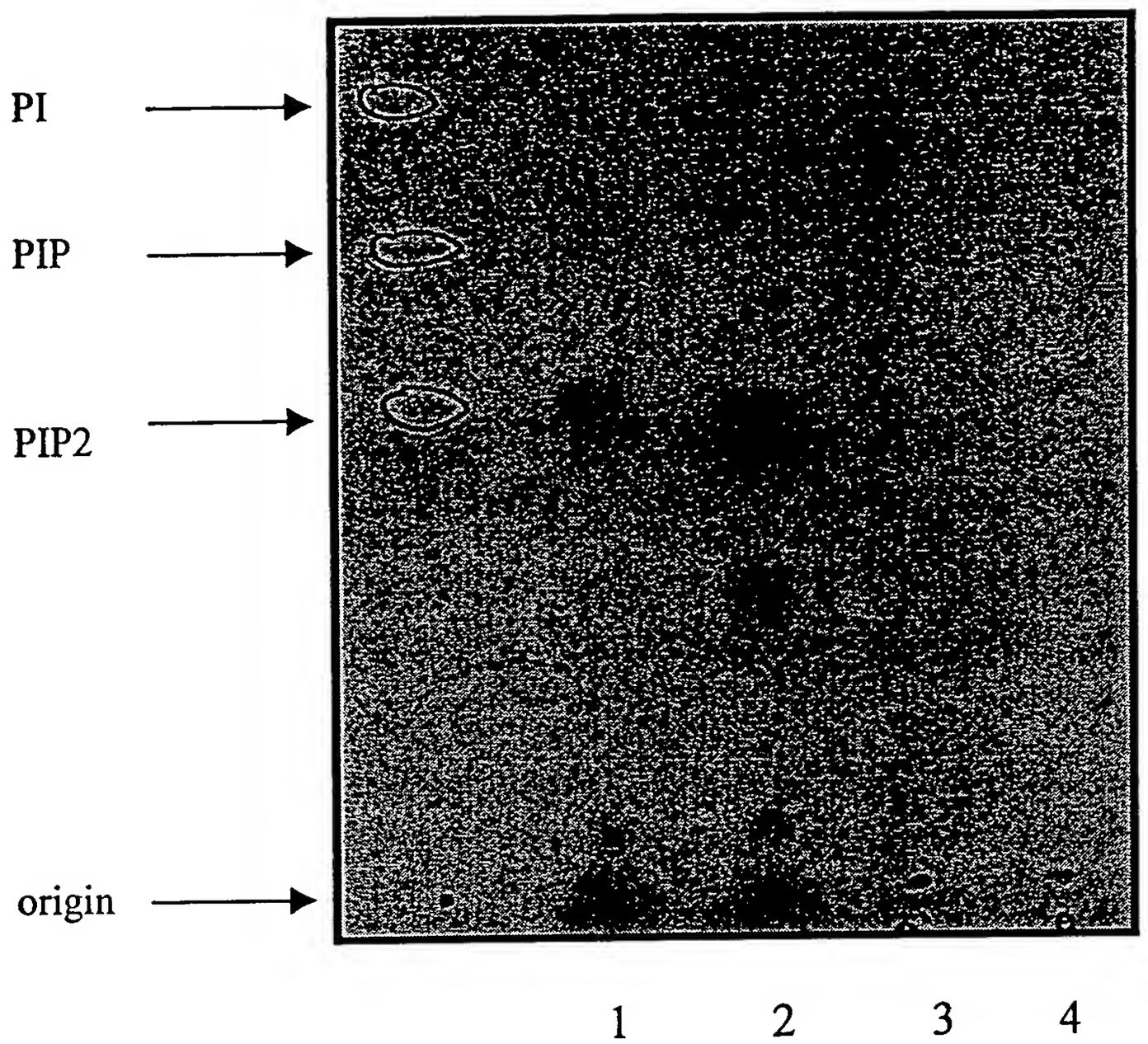

FIG. 10

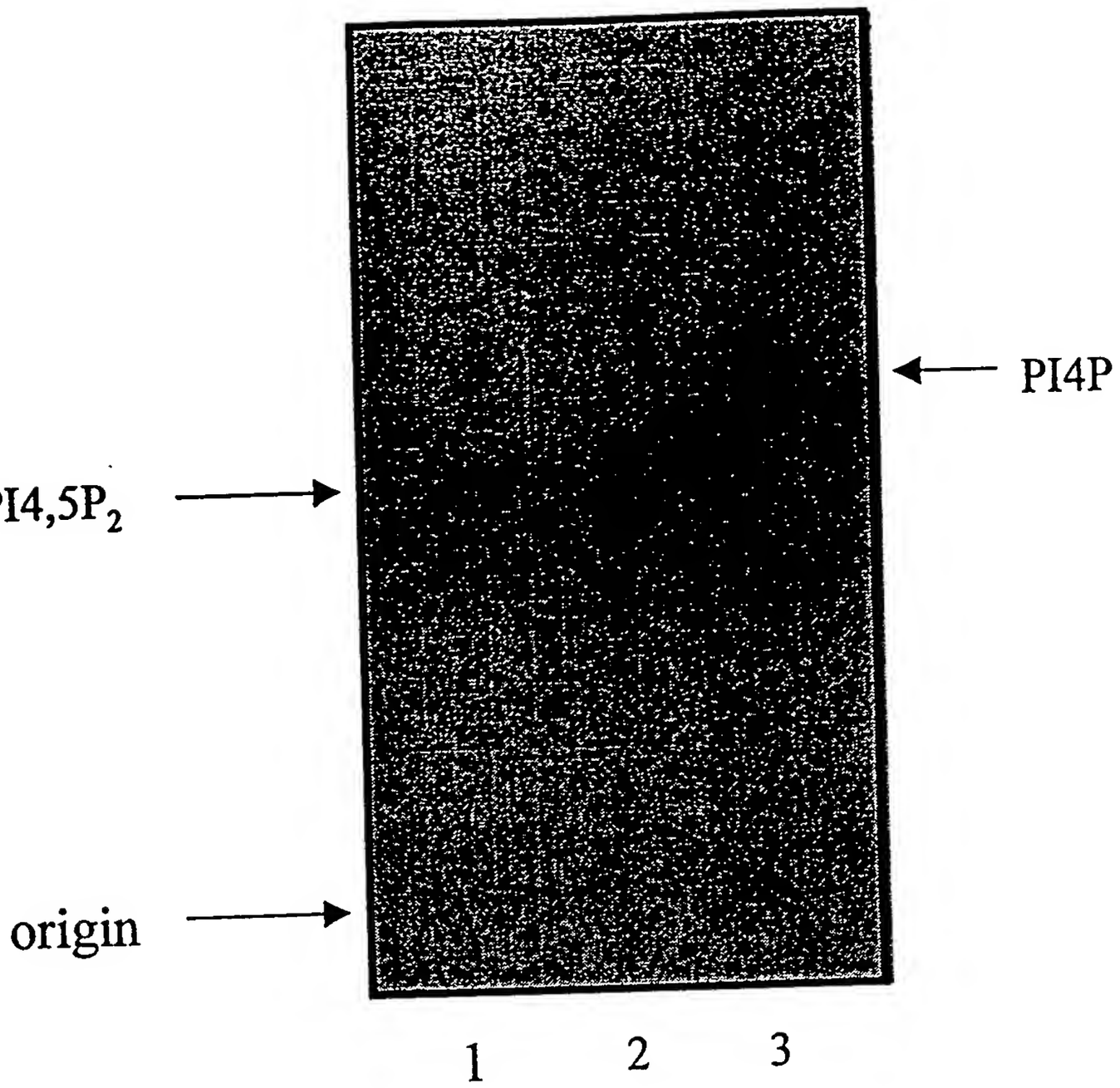

FIG. 11A

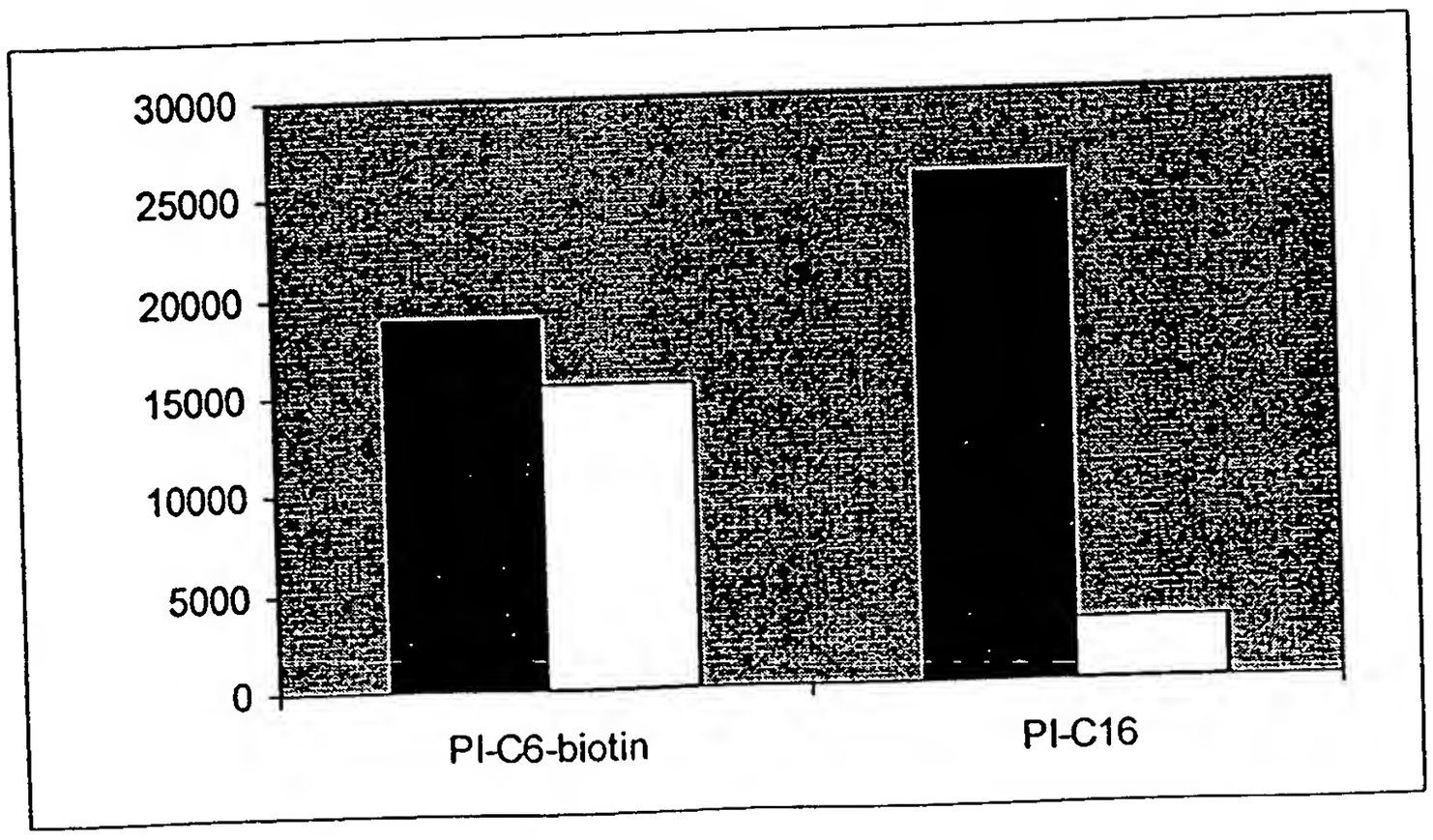

FIG. 11B